Distribution of Polychlorinated Biphenyls and Chlorinated Pesticide Residues in Trout in the Sierra Nevada

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ABSTRACT

Organochlorine compounds are known to be atmospherically transported to long distances from their original sources. To understand the influence of California's Sierra Nevada range on the air transport and subsequent distribution pattern of some of these residues within the range, we have chosen salmonid fish as an indicator species. Fish were collected from 10 locations throughout the northern and central Sierra Nevada and polychlorinated biphenyl (PCB), toxaphene, chlordane, and DDT [1,1,1-trichloro, 2,2′-bis (*p*-chlorophenyl) ethane] residues in muscle tissue were analyzed. Rainbow trout (*Oncorhynchus mykiss*) were found in all sampling locations, and therefore analyses mainly focused on this species. When similar-sized rainbow trout samples from several similar oligotrophic, high-altitude lakes and streams were compared, it became apparent that altitude is one of the factors affecting the residual levels of PCB (r^2 = 0.882), but not for total DDT, toxaphene, or chlordane in trout. Analysis of correlations among these four organochlorine compound residue groups indicated that there are modest correlations in patterns of distribution between chlordane vs. toxaphene (r^2 = 0.345), and chlordane vs. total DDT (r^2 = 0.239), but toxaphene residues are not correlated with PCB or total DDT. In view of significant correlation to the altitude it is concluded that PCB residue in rainbow trout is a good monitoring tool for studying the effect of high-altitude mountain ranges on the long-range transport and distribution of those persistent pollutants.

It is well recognized that certain organochlorine compounds such as PCBs, toxaphene, and DDE [1,1-dichloro, 2,2′-bis (*p*-chlorophenyl) ethylene] are transported in the atmosphere from sources in temperate regions to distant locations such as the Arctic and Antarctic (Buser et al., 1993; Schmitt et al., 1990; Zell and Ballschmitter, 1980). Over time, significant amounts of these chemicals may be deposited through wet and dry deposition. For instance, most of the organochlorine compound burden in Isle Royale, an island in Lake Superior, has been attributed to atmospheric sources, based on the residue concentrations in fish (Swackhamer and Hites, 1998). Aside from the work of Blais et al. (1998), the influence of high mountain ranges on the distribution patterns of these organochlorine residues in the North American continent has not been well investigated, though there are reports indicating the presence of some organochlorine compound residues in remote mountain lakes (Heit et al., 1984). Previously we found that samples of lake trout (*Salvelinus namaycush*) from Lake Tahoe contain quantifiable amounts of PCBs (Datta et al., 1999). It has also been documented by Cory et al. (1970) that relatively large amounts of DDT and its metabolites are present in mountain yellow-legged frogs (*Rana muscosa*) indigenous to high elevations in the Sierra Nevada.

In spring, summer, and fall, prevailing winds blow from west to east across California's Central Valley and continue up the western slope of the Sierra Nevada (Hayes et al., 1984). These regional wind patterns are due to the jet stream and up-slope/down-slope air mass movements associated with daytime to nighttime temperature differentials, and such wind patterns result in movements of contaminated air masses from the Central Valley into the Sierra Nevada (Cahill, 1989). This extensive, north–south mountain range flanks the eastern edge of the entire state of California, and contains some of the highest peaks in the lower continental United States, including many over 4000 m (13 100 ft). In crossing the range, rising air masses release much of their moisture as precipitation on the western slope, resulting in fairly dry conditions on the eastern side of the crest (McIlyeen, 1992). This "rain shadow effect" might theoretically result in diminished deposition of pollutants originating from California on the eastern slope (or side) of the Sierra Nevada; such a phenomenon could suggest an air "scrubbing" effect by high alpine ranges of regional and/or global pollutants. Our main objective is to first assess the residual concentrations of organochlorine compounds in salmonid fish samples from several selected lakes and streams in this region, and second to evaluate the influence of altitude, locations, and human activities on organochlorine distribution patterns. Source locations of two of the main pollutants within California are geographically distinct. Toxaphene had been used heavily on cotton as a 1:1 mixture with DDT in the Central Valley, thus its major reservoir is confined to the cotton-growing area of between Fresno and Bakersfield. Sources of PCBs may be more diffuse, but the most populated San Francisco Bay to Sacramento corridor region (approximately 250 km north of the cotton-growing area) is the likely major source in central and northern California. In light of the very low levels of organochlorine residues expected in air and water in the remote areas of the Sierra Nevada, our basic strategy has been to use trout as an initial indicator species to monitor the overall pattern of PCB and toxaphene distribution in aquatic organisms in the Sierra Nevada. This approach does not directly establish that the source of pollution is atmospheric, nor does it immediately show that a quantitative relationship exists between the level of these pollutants found in fish and the total quantity

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Abbreviations: ECD, electron capture detector; GC, gas chromatography; MS, mass spectrometry; PCB, polychlorinated biphenyl; SIM, select ion monitoring.

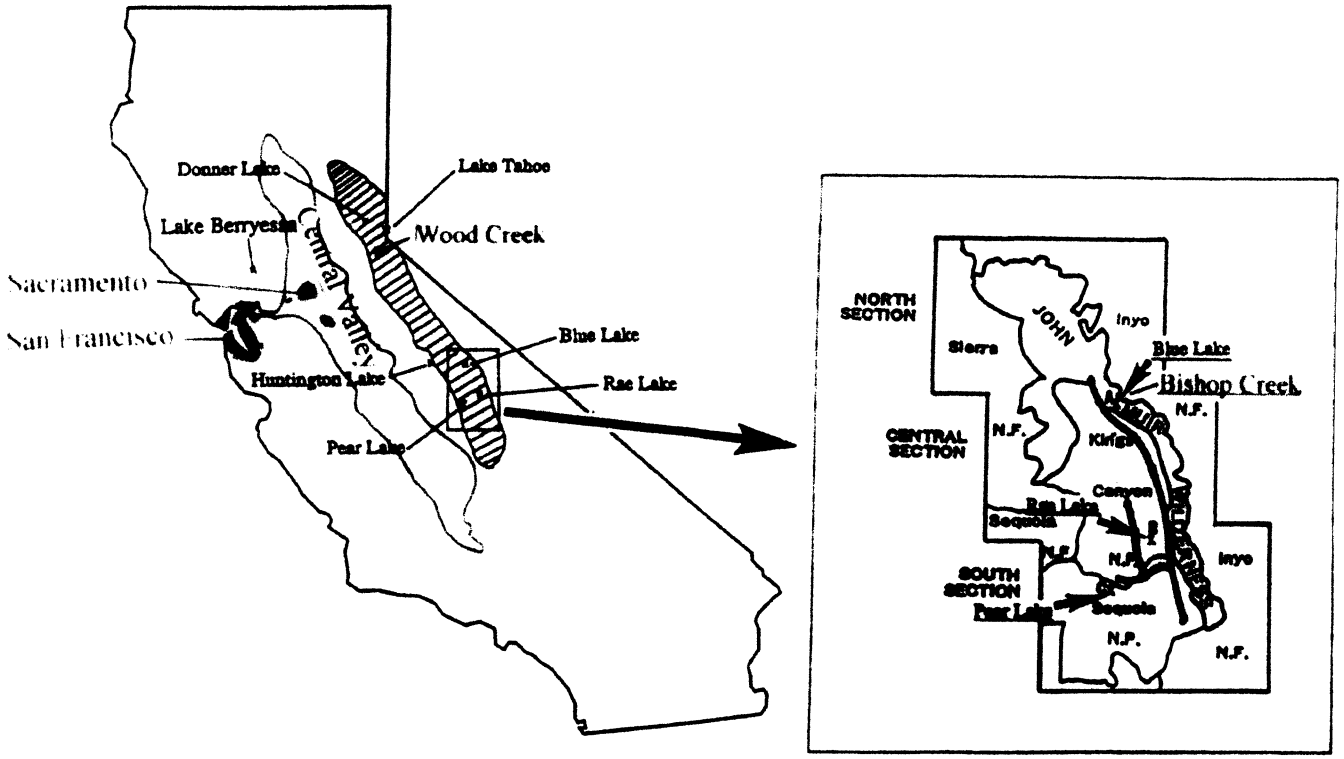

Fig. 1. Sierra Nevada fish sample locations and the general range of our main study area (indicated by shaded area) for high-altitude rainbow trout sampling, and an expanded map showing three sites in the central Sierra Nevada area where trout samples were collected (see arrows). Bold lines indicate high mountain divides between 3663 and 4273 m elevation. Site A: Blue Lake at 37° 8′ N, 118° 37′ 30° W directly east of Mt. Mendel (4179 m) and Mt. Darwin (4240 m), a 4-h hike from Lake Sabrina. Site B: Rae Lake, Sixty Lakes Basin at 36° 57′ N, 188° 23′ W, directly east of Mt. Gardiner (3959 m) and west of Black Mountain (3970 m), a 2-d hike from Onion Valley (2803 m) through Kearsarge (3526 m) and Glenn Pass (3656 m). Site C: Pear Lake at 36° 37′ N, 118° 40′ W, directly west of Elizabeth Pass (3611 m), 3 h from Wolverton.

of pollutant inputs into these bodies of water, because other factors such as organic carbon content in sediments and trophic status may affect fish and water distributions directly (Larsson et al., 1992). Nevertheless, the basic lake characteristics of some high-altitude sampling sites are similar, and they are located in areas far removed from human habitation and activities, and at high elevations that are usually inaccessible to motorized boats and vehicles (Fig. 1, right panel). Therefore, at least in these locations, direct anthropogenic pollutant inputs may be considered to be unlikely. The locations of collection sites in relation to the entire Sierra Nevada range are shown in Fig. 1.

MATERIALS AND METHODS

Biological Materials

All fish were collected during the summer months of 1995 and 1996. Fish taken from lakes accessible to motorized boats (Lake Tahoe, Huntington Lake, Donner Lake, and Lake Berryessa) were caught by hook-and-line with artificial lures from a research boat bearing a gasoline-powered motor. At sites inaccessible to motorized boats (Blue Lake, Pear Lake, and Rae Lake), fish were caught by hook-and-line with artificial flies. Fish caught in Lake Tahoe were lake trout (*Salvelinus namaycush*) and Kokanee (*Oncorhynchus nerka*), and those from Donner Lake were Kokanee only. Rainbow trout collected in national parks were from locations where no motorized boats were permitted, and no planting of rainbow trout has been recorded since 1975 (James Hopelain, California Department of Fish and Game, personal communication, 1986). All Sierra Nevada fish were collected from lakes and streams between 1830 m (6000 ft) and 3500 m (11 500 ft) in elevation.

Fish were killed with a blow to the head and placed on ice (or snow pack) within 2 h of collection. Upon returning to the laboratory, fish were transferred to a −20°C freezer and maintained at this temperature until subsampled and analyzed.

Experimental Sample Preparation and Analytical Extraction

Sample preparation was similar to that of Stanley and LeFavoure (1965) as modified by Luckas (1990). One-gram portions of fish muscle were excised proximal to the dorsal fin and digested in 2 mL of an acetic acid and perchloric acid mixture (1:1, v/v at 70°C for 3 h), and then extracted twice with 2 mL of *n*-hexane. The extracts were mixed with 2 mL of concentrated sulfuric acid to remove fat and nonpersistent compounds. This step was repeated until the sulfuric acid phase became transparent (Murphy, 1972; Tarhaned et al., 1989). Purified hexane extracts were reduced to 1 mL under a N_2 gas stream and cleaned up using a silica gel column chromatography (Ribick et al., 1982) with a scaled down modification. Silica gel columns were prepared by placing anhydrous Na_2SO_4 in a disposable pipette (146 mm × 5.5-mm i.d.) plugged with glass wool. Half-gram volumes of silica gel (200–400 mesh, 60 Å; Aldrich, St. Louis, MO), activated at 170°C overnight, were added and topped with anhydrous Na_2SO_4. The columns were washed with 3 mL of hexane before adding the sample extracts. Columns were eluted with 3.2 mL of 0.3% benzene in hexane (v/v) to elute PCBs and most of the DDE (designated as the PCB fraction), and then washed with 25% diethyl ether in hexane (v/v) to elute toxaphene, DDT, and its metabolites including residual DDE, *cis*- and *trans*-chlordanes,

oxychlordane, and *trans*-nonachlor (designated as the toxaphene fraction). The eluates were treated with concentrated sulfuric acid again to eliminate analytical interferences. The solvents were exchanged into isooctane and taken to a 1-mL final volume before analysis by gas chromatography. Further details of sample cleanup and verification of individual organochlorine compound residues through gas chromatography (GC)–mass spectrometry (MS) running in select ion monitoring mode (SIM) have already been published by this laboratory (Datta et al., 1999).

Toxaphene reference standard was obtained from the USEPA repository (Research Triangle Park, NC) and Aroclor reference standards (Aroclor 1016, 1221, 1232, 1242, 1248, 1254, 1260, and 1262) were purchased from PolyScience (a division of Preston Industries, Niles, IL). The *p,p′*-DDT, *p,p′*-DDE, *p,p′*-DDD, *trans*- and *cis*-chlordanes, oxychlordane, and *trans*-nonachlor reference standards were obtained from Velsicol Chemical Co. (Chicago, IL). Decachlorobiphenyl (DCB) standard, which was used as a spiking compound for recovery tests, was purchased from Axact Standards (Commack, NY). For identification of major peaks of toxaphene, purified and NMR-certified toxicants A1 and A2 (Matsumura et al., 1975) and toxicant B (Chandurkar and Matsumura, 1979; Nelson and Matsumura, 1975) were used. All solvents were either pesticide grade or high-resolution GC grade. Acetic acid (glacial), perchloric acid (69–72%), and sulfuric acid were all trace-metal grade from Fisher Scientific (Hampton, NH). Recoveries from spiked samples were 95.8% (DCB, spiked level = 20 μg kg^{-1}), 98.2% (Aroclor 1260, spiked level = 200 μg kg^{-1}) for PCBs, and 86.4% for toxaphene (spiked level = 200 μg kg^{-1}).

Analysis

All GC analyses were performed with a Varian (Palo Alto, CA) 3400 gas chromatograph equipped with a ^{63}Ni electron capture detector (ECD) and split injection at a ratio of 1:10. A 30 m × 0.25-mm i.d. DB-5 (0.25 μm film thickness; J&W Scientific, Folsom, CA) was used for routine analyses. Other GC conditions used were as follows: N_2 carrier gas, 34 cm s^{-1} (linear velocity); N_2 make-up gas, 25 mL min^{-1}; injection port, 280°C; and detector, 360°C. Initial column temperature was held at 200°C for 1 min and then time programmed to 280°C at 8°C min^{-1}. Data were collected with a Hewlett-Packard (Palo Alto, CA) 3390A integrator.

Each organochlorine compound was confirmed by operating at a relatively slow GC column temperature program with an initial temperature held at 200°C for 5 min to eliminate solvent peaks, then programmed to 280°C at 2°C min^{-1} on both DB-5 (described above) and DB-1701 (22 m × 0.25-mm i.d., 0.25-μm film thickness; J&W Scientific). A GC–MS confirmation of these compounds also was performed, as reported in Datta et al. (1999).

Total DDT concentrations were calculated by summing *p,p′*-DDT, *p,p′*-DDE, and *p,p′*-DDD concentrations. For PCB concentrations, early eluting GC peaks matched the Aroclor 1254 standard and were calculated as Aroclor 1254, and other GC peaks that matched the Aroclor 1260 standard were calculated as Aroclor 1260. The sum of Aroclor 1254 and 1260 values was expressed as the total PCB concentration. Quantification of "estimated total toxaphene" was completed using the method of Gooch and Matsumura (1985). Total chlordane values were calculated by summing *cis*- and *trans*-chlordanes, oxychlordane, and *trans*-nonachlor concentrations.

Toxaphene fractions from Lake Tahoe and Huntington Lake samples were subjected to GC–MS–SIM to confirm the presence of toxaphene residues. The GC–MS–SIM analyses were not applied to samples other than those from Lake Tahoe and Huntington Lake, since the quantities of organochlorine compound residues available from other locations were very small and, therefore, below detection limits. The GC–MS system (VG Trio-2 quadruple mass spectrometer with a Hewlett-Packard 5890 GC) was equipped with a DB-1 fused silica capillary column (30 m × 0.25-mm i.d., 0.25-μm film thickness; J&W Scientific) and was operated in the electron impact (EI) ionization mode with an ionization voltage of 70 eV. Conditions for GC–MS analysis were as follows: splitless injection mode; He carrier gas at 35 cm s^{-1} (linear velocity); injection port, 275°C; transfer line, 285°C; and ion source, 150°C. The instrument was set to monitor atomic mass of *m*/*z* 158.9 (C_7! $H_5C1_2^+$) and its isotope at *m*/*z* 160.9, which are general fragments of toxaphene components that are not contributed significantly to by other organochlorine compounds (Ribick et al., 1982; Gooch and Matsumura, 1985).

Quality Assurance–Quality Control

Data presented in this paper were not corrected for recovery. Two types of blanks were used to ascertain the absence of interfering contaminants in this assay procedure. One is "procedural blanks," which were run parallel to each batch of samples but without biological materials to check possible contaminants from solvents, columns, and any other materials used for the above purification procedure. The second blanks were provided by trout samples from Rae Lake, which contained very low organochlorine compound levels. These two sets of blanks showed that the procedures we used were appropriate, and in no case was the presence of interferences detected.

Statistical Treatment

Fish collected from various sites were initially compared through an analysis of covariance (ANCOVA) to examine the possibility that the body weight would affect the level of organochlorine compound residues. Additional analyses of variance (ANOVAs) were performed to investigate the effect of site elevation, latitude, longitude, drainage basin aspect, and species on fish tissue organochlorine concentrations. Fish sites were assigned to either east-facing or west-facing catchment basins, as derived by inspection of contour features and drainage patterns on USGS 7.5-min topographic quadrangles. All data transformations and statistical tests were conducted using StatView 512+ (SAS Institute, 2002), except for the final regression analysis on the influence of altitude data, which was conducted using SYSTAT Version 10.2 (Systat Software, 2002). The R^2 values represent the coefficient of determination. For instance, R^2 of 0.882 indicates that 88.2% of the variability of one variable (e.g., PCB residue) can be explained by the other variable (e.g., altitude). The *p* value is the probability that the correlation observed can take place by chance alone.

RESULTS

Qualitative Verification of Organochlorine Residues Recovered from Fish Samples

The main purpose of this qualitative assessment of organochlorine compound residues is to make sure that these mixtures of organochlorine compound components are properly grouped into each class of organochlorine compounds. This study is not meant for toxic congener analysis, since the main objective of this project is to study the distribution of organochlorine compounds, not their toxic effects. Chromatograms of residue patterns were analyzed first in large lake trout

samples from Lake Tahoe in comparison with several PCB standards, since they offered a sufficient quantity of residues. The results of GC pattern analyses indicated that PCB congeners from lake trout extracts contained many peaks matching those of Aroclor standards (data not shown). From the viewpoint of the number of exact peak position matching, Aroclor 1254 and 1260 were the best diagnostic indicator, since the later eluting peaks were easier to recognize and match precisely. To reach this conclusion, we compared Aroclor 1016, 1221, 1234, 1242, 1248, 1254, 1260, and 1262. On the other hand, there are some early eluting peaks in the lake

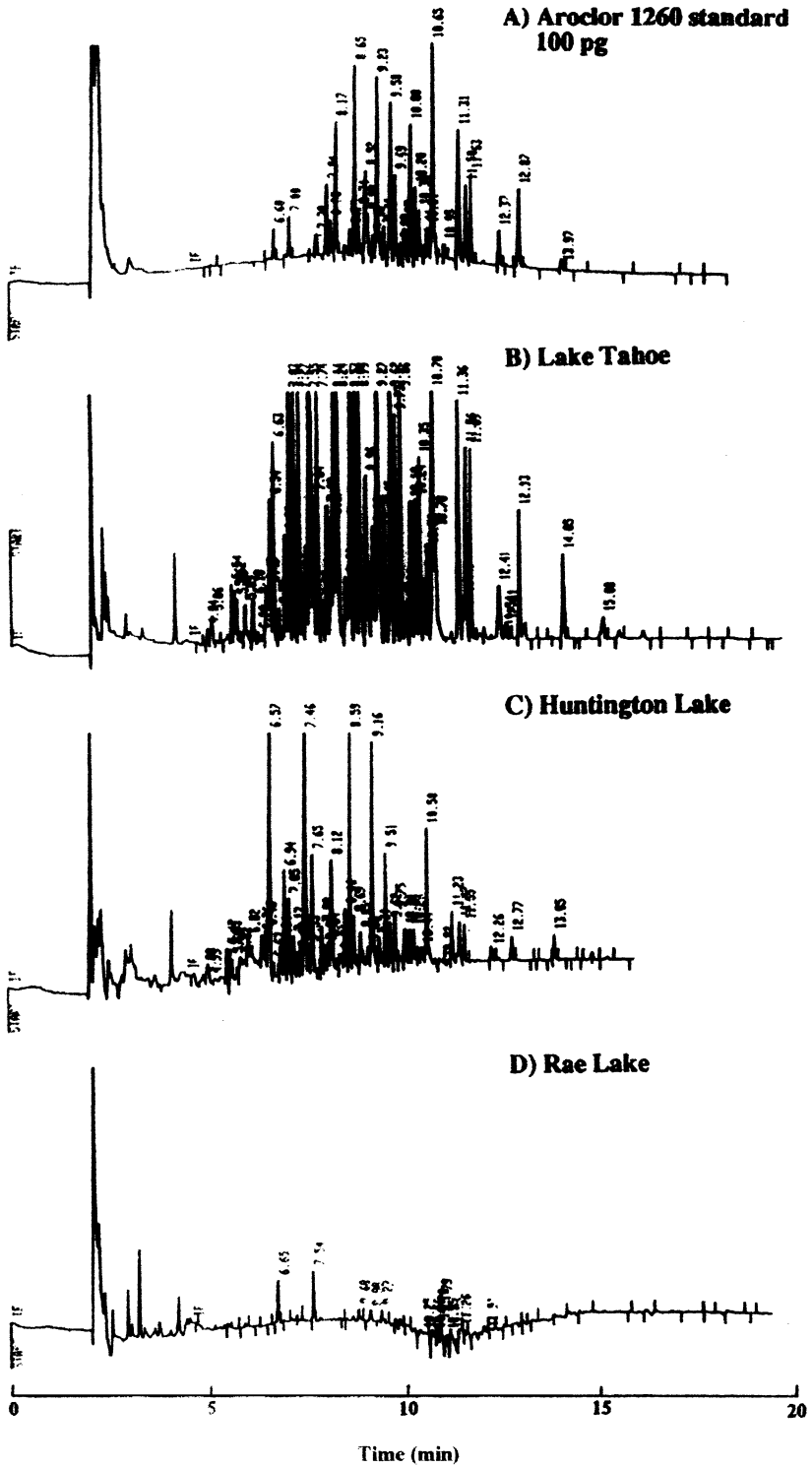

Fig. 2. Examples of DB5 capillary gas chromatography (GC)–electron capture detector (ECD) patterns of standard polychlorinated biphenyl (PCB) samples and an example of residues found in a PCB fraction obtained from muscles of a lake trout sample from Lake Tahoe, Sierra Nevada.

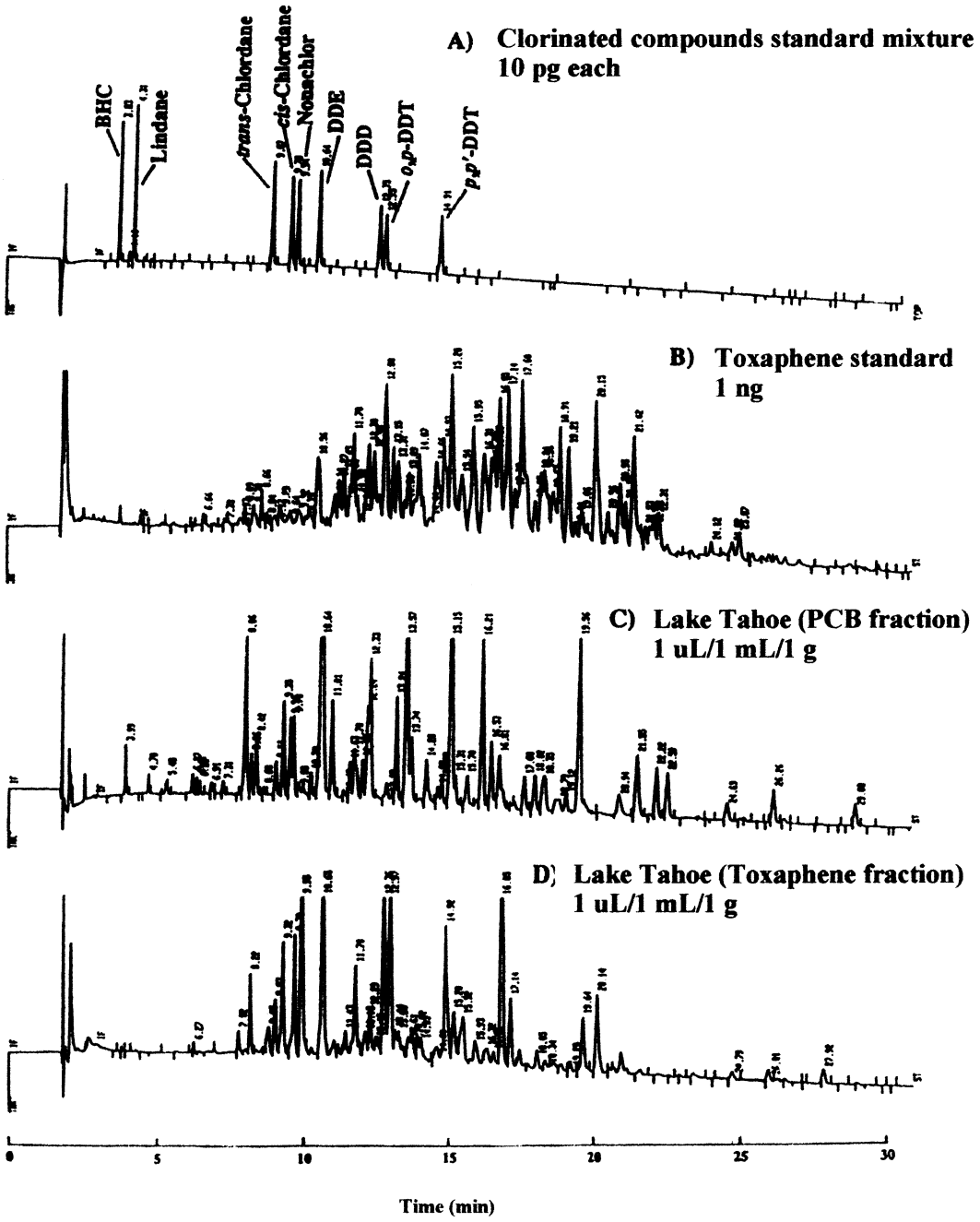

Fig. 3. Examples of DB5 capillary gas chromatography (GC)–electron capture detector (ECD) chromatograms of standard toxaphene, other chlorinated pesticides, and residues found in toxaphene fraction obtained from muscles of lake trout samples from Lake Tahoe, Sierra Nevada. See Table 4 for the description of lake trout.

trout samples that are well matched to Aroclor 1248 (data not shown). A lake trout sample from Lake Tahoe was also analyzed using GC–MS to verify that the fragmentation patterns were consistent with PCB congeners (data not shown). A GC–MS confirmation of toxaphene in lake trout from Lake Tahoe and rainbow trout from Huntington Lake was achieved using *m*/*z* 340 and 159 as characteristic ions and *m*/*z* 342, 344, and 161 as indicators of chlorine isotope ratios, as well as peak matching to the authentic toxaphene standard on a DB-1 column. The PCB residue congener patterns among rainbow trout samples from high-elevation lakes were also compared (Fig. 2). The results showed that the sample from Rae Lake is almost devoid of PCB residues. The samples from Lake Huntington were qualitatively very similar to that from Lake Tahoe, though relatively fewer PCB congener peaks were present in the former sample. In a similar fashion, the peak patterns of toxaphene fraction from the same lake trout sample from Lake Tahoe were compared with those of PCB fraction and to a standard sample of toxaphene and those of several organochlorine pesticides (Fig. 3). The results show that toxaphene fractions contain compounds of which peaks match very well to those of standard toxaphene, along with the peak

Table 1. Summary of geographic characteristics of collection sites.

Site (abbreviation)	Elevation	Latitude		Longitude		Distance from nearest point of the 100-m Central Valley contour
		UTM, northern	Degrees, northern	UTM, eastern	Degrees, western	
	m					km
Rae Lake (RL)	3212	4074483	36.80795	11S 375030	118.40098	120.84
Blue Lake (BL)	3165	4116507	37.18396	11S 356292	118.61898	129.48
Pear Lake (PL)	2910	4051856	36.60056	11S 350912	118.66685	74.40
SF Bishop Creek (SB)	2452	4123927	37.25147	11S 360624	118.57158	140.05
Woods Lake Creek (WL) at Winnemucca Lake outlet	2739	4284411	38.66989	11S 239181	119.99787	99.64
Lake Tahoe (LT)	1899	4328400	39.06721	10S 755907	120.04214	107.85
Huntington Lake (HL)	2118	4123835	37.24073	11S 305320	119.19480	84.21
Donner Lake (DL)	1808	4356106	39.32221	10S 735940	120.26301	101.33
Lake Berryessa (LB)	133	4272058	38.59450	10S 566759	122.23340	–
Union Valley Reservoir (U)	1476	4305624	38.87093	10S 724061	120.41732	72.35

Table 2. Residues of polychlorinated biphenyls (PCBs), toxaphene, total DDT, and chlordane in muscles of rainbow trout collected from altitudes between 2452 and 3212 m.

Site	Fish weight	PCBs			Toxaphene			Total DDT			Chlordane		
		Value	Mean	SD	Value	Mean	SD	Value	Mean	SD	Value	Mean	SD
	g	μg kg^{-1} (wet wt.)											
							Rae Lake (RL)						
R1	68	1.03	1.15	0.13	1.75	1.75	0.36	0.89	1.24	0.38	0.22	0.26	0.05
R2	83	1.12			2.11			1.65			0.32		
R3	65	1.29			1.40			1.17			0.25		
							Blue Lake (BL)						
B1	57	2.17	2.45	1.53	6.38	4.23	2.39	1.68	2.13	1.13	0.59	0.46	0.20
B2	90	1.50			1.90			1.25			0.21		
B3	74	1.92			4.19			2.02			0.56		
B5	66	1.10			2.63			0.94			0.35		
B6	131	2.64			2.48			2.88			0.30		
B7	27	5.37			7.82			3.98			0.72		
							Pear Lake (PL)						
P1	57	2.08	3.28	1.34	1.84	1.84	0.38	6.21	9.99	4.07	0.45	0.53	0.12
P2	68	3.69			2.30			8.22			0.68		
P3	88	5.12			1.61			14.44			0.50		
P4	59	3.65			2.11			14.30			0.63		
P5	62	1.85			1.36			6.78			0.40		
							Wood Creek (WC)						
C1	59	1.43	3.73	3.99	2.52	1.80	0.65	3.05	2.55	0.50	0.58	0.38	0.15
C2	41	2.09			2.11			2.39			0.36		
C3	35	9.70			1.03			2.83			0.22		
C4	40	1.71			1.52			1.93			0.37		
							South Fork Bishop Creek (SB)						
J1	38	6.22	8.28	4.98	2.86	2.64	0.47	2.82	2.81	1.41	0.51	0.46	0.09
J2	107	3.60			3.20			1.38			0.35		
J3	73	8.08			2.34			2.32			0.43		
J4	93	15.22			2.17			4.72			0.54		

positions of toxicant A and B (Gooch and Matsumura, 1985) (Fig. 3). The only peak that appeared consistently in both PCB and toxaphene fractions was *p,p′*-DDE (e.g., peak 10.63–10.65; Fig. 3).

Comparison of Residue Concentrations among Different Groups of Fishes

The location and the altitude of each sampling site are listed in Table 1. The location, the concentrations of organochlorine compound residues found, and the weights of rainbow trout samples are shown in Table 2 and 3 along with those of other trout species in Table 3 and 4. It was found that there were significant differences in organochlorine compound concentrations in different species even within the same water body (Tables 3 and 4). Therefore, we selected rainbow trout as the primary indicator species for comparisons of organochlorine compound levels among different locations. The relationships between the body weight and the levels of four organochlorine compound residues in all rainbow trout samples were examined next (Table 2 and 3). The results indicate that in general, there was no significant correlation of analyte to body weight for any of the organochlorine compounds studied, among samples that were less than 315 g (Fig. 4). There was a correlation of PCB residues and body weight, (slope of 0.021, $R^2 = 0.46$, $n = 33$) when samples were taken from all fish, including those greater than 315 g. The lack of correlation between body weight and residue levels in fish less than 315 g was particularly apparent in the case of toxaphene, total DDT, and chlordane. Therefore, in all subsequent data analyses, we expressed the raw data in μg kg^{-1} (or ppb) without any attempt for normalization for their body weights. Furthermore, it was neces-

Table 3. Residues of polychlorinated biphenyls (PCBs), toxaphene, DDT, and chlordane in muscles of rainbow trout collected from lakes below altitudes of 2200 m along with reference samples.

Site	Fish weight	PCBs			Toxaphene			Total DDT			Chlordane		
		Value	Mean	SD	Value	Mean	SD	Value	Mean	SD	Value	Mean	SD
	g	μg kg^{-1} (wet wt.)											
							Donner Lake (DL)						
D1	225	4.21	5.91	2.40	1.40	1.96	0.78	2.47	3.71	1.75	0.41	0.61	0.28
D2	142	7.60			2.51			4.94			0.80		
							Huntington Lake (HL)						
H1	1700	121.37	52.79	55.87	69.34	24.30	31.05	78.29	30.55	35.29	18.91	7.27	8.64
H2	450	75.29			20.45			36.19			8.76		
H3	330	9.17			4.40			4.46			0.83		
H4	150	5.34			3.02			3.27			0.57		
							Lake Berryessa (LB)						
L1	683	9.87	10.82	4.80	3.69	2.42	2.02	26.48	13.01	8.83	1.87	1.08	0.87
L2	315	19.03			5.38			17.20			2.16		
L3	261	8.86			1.17			6.23			0.56		
L4	203	6.46			0.82			5.62			0.26		
L5	291	9.88			1.06			9.50			0.53		
							UCD Aquaculture (AQ)						
U1	276	4.71	4.42	1.38	1.89	2.96	1.35	1.77	2.00	0.94	0.52	0.40	0.17
U2	167	2.67			2.82			1.74			0.37		
U3	182	4.28			4.91			3.34			0.54		
U4	177	6.01			2.23			1.14			0.18		
							Union Valley (U)						
Un1	268	3.97	3.63	0.31	3.22	5.06	1.83	2.53	2.81	0.39	0.48	0.44	0.04
Un2	307	3.54			6.87			3.25			0.42		
Un3	221	3.38			5.09			2.64			0.41		
							Commercial fish						
S1	395	29.44			9.67			8.29			2.10		
S2	572	37.40			12.59			13.59			5.11		

sary to limit the scope of interlocation comparisons to samples to only rainbow trout smaller than 315 g, since at high altitudes only small-sized trout were available. The results of the residue survey on rainbow trout samples from high-altitude lakes and streams indicated that those collected from Rae Lake, in the Sixty Lakes Basin region of King's Canyon National Park, showed the lowest levels of residues of all four organochlorines analyzed. Fish collected from Pear Lake, a lake of similar size and elevation but on the western slope of the Sierra, had much greater levels of PCBs but a comparable level of toxaphene. The PCB concentrations in trout from Blue Lake, which lies in eastern King's Canyon National Park and at a comparable elevation and latitude to Pear Lake, were intermediate in value yet these fish had slightly higher levels of toxaphene compared with fish from Pear Lake. The results of this set of analyses indicate that altitude is not the only factor that affects the concentrations of all organochlorine compound residues. To see whether the residue pattern in streams is different from lakes, rainbow trout from two small streams (see Table 1) at 2700 m (Wood Creek) to 2452 m (south fork of Bishop Creek) were studied. The results showed (Table 2) similar levels of organochlorine compound residues. In the case of the latter sample the level of toxaphene was comparable with that in trout from nearby Blue Lake, but its levels of PCBs were higher in the Bishop Creek samples.

Table 4. Residues of polychlorinated biphenyls (PCBs), toxaphene, DDT, and chlordane in muscles of other species of fish from Donner Lake and Lake Tahoe along with reference samples.

Species	Fish weight	PCBs			Toxaphene			Total DDT			Chlordane		
		Value	Mean	SD	Value	Mean	SD	Value	Mean	SD	Value	Mean	SD
	g	μg kg^{-1} (wet wt.)											
							Donner Lake (DL)						
D3, Kokanee	228	56.16	68.46	33.32	21.13	29.51	11.97	13.71	18.54	7.84	14.63	19.82	8.04
D4, Kokanee	281	106.19			43.22			27.58			29.08		
D5, Kokanee	207	43.04			24.19			14.32			15.74		
							Lake Tahoe (LT)						
T1, lake trout	6600	547.84	222.61	201.47	307.02	187.95	112.80	334.60	119.47	129.05	140.37	83.17	56.41
T2, lake trout	1550	125.88			212.81			72.01			115.00		
T3, lake trout	3900	287.05			279.38			139.26			115.69		
T4, lake trout	1500	66.23			73.80			18.25			22.94		
T5, lake trout	2300	86.36			66.73			33.21			21.86		
							Saskatchewan						
Sa1, lake trout	–	23.53	23.53		23.74	23.74		21.70	21.70		6.94	6.94	
							Commercial fish						
S3, king salmon	372	162.31			68.12			95.73			18.69		

In general, samples from Rae Lake showed by far the lowest concentrations of all organochlorine compounds, whereas those from Huntington Lake had the largest concentrations. One clear exception was the samples from Pear Lake, which had relatively high total DDT and suggest either increased deposition or total DDT contamination at this site is specific (Table 2). Total chlordane levels were between those observed for total DDT and toxaphene. They also showed some alpine effects, but they were not as pronounced as PCBs or total DDT. Among the three high-elevation lakes, the ranked order of chlordane concentrations was Pear > Blue > Rae, with an inverse correlation to elevation (Table 2). Organochlorine residue data from fish samples from lower-altitude lakes that are accessible to motorized boats and closer to human habitation are shown in Table 3 and compared with the UC Davis aquaculture and commercial fish data. In general, samples from lower elevations show increased concentrations of organochlorine compound residues. Particularly noticeable was the high organochlorine compound residues in the heaviest rainbow trout samples (450 g) from Huntington Lake and Lake Berryessa.

In the next data analysis, the potential relationships between concentrations of these four organochlorine compounds in all individual rainbow trout samples less than 315 g were assessed (Fig. 5). The results of this investigation showed that chlordane residue levels are loosely correlated, all in positive directions, to those of toxaphene (Fig. 5E), total DDT (Fig. 5F), and to a lesser extent to those of PCB (Fig. 5C). Also, there was a slight positive correlation between total DDT and PCB (Fig. 5B). Toxaphene residue levels, on the other hand, did not show any positive correlations to either PCB (Fig. 5A) or total DDT (Fig. 5D). These results support the notion that transport and distribution processes affect PCB, total DDT and chlordane in a similar manner, but the mechanism affecting toxaphene may be different.

The effects of altitude on organochlorine compound residue concentrations in all fish samples less than 315 g were also examined (Fig. 6). Concentrations of PCB showed a noticeable altitude-dependent decline in residue levels (covariance = −2164.9 and correlation = −0.465). Surprisingly, the other three organochlorine compounds did not show any effect of altitude.

To aid visualization of quantitative differences among fish samples from different elevations in the level of residues of these organochlorines, ANOVA analyses were conducted (Table 5). Residues of PCB and DDT from Lake Berryessa (LB, the lowest elevation) samples are statistically different from others, and that those of Rae Lake (RL, the highest) are different from Huntington (HL), and the south fork of Bishop Creek (SF) and DDT residue of Pear Lake (PL) samples are different from several others (all locations are in California).

Since the pattern observed for PCB–altitude relationship (Fig. 6A) may simply be due to the body weight difference between rainbow trout from lowlands and those from highlands, we reanalyzed only those samples collected from the high-altitude locations where there was no chance of large trout size differences and significantly less anthropogenic input due to limited access. We found no correlation between the size of rainbow trout and the elevation among these samples (Fig. 7B). It was also possible to circumvent the question of differences in lake characteristics by selecting samples collected at elevations between 2400 and 3300 m. Elevation-related variations in residue concentrations indicated that PCB residues (Fig. 7C) are most affected by alti-

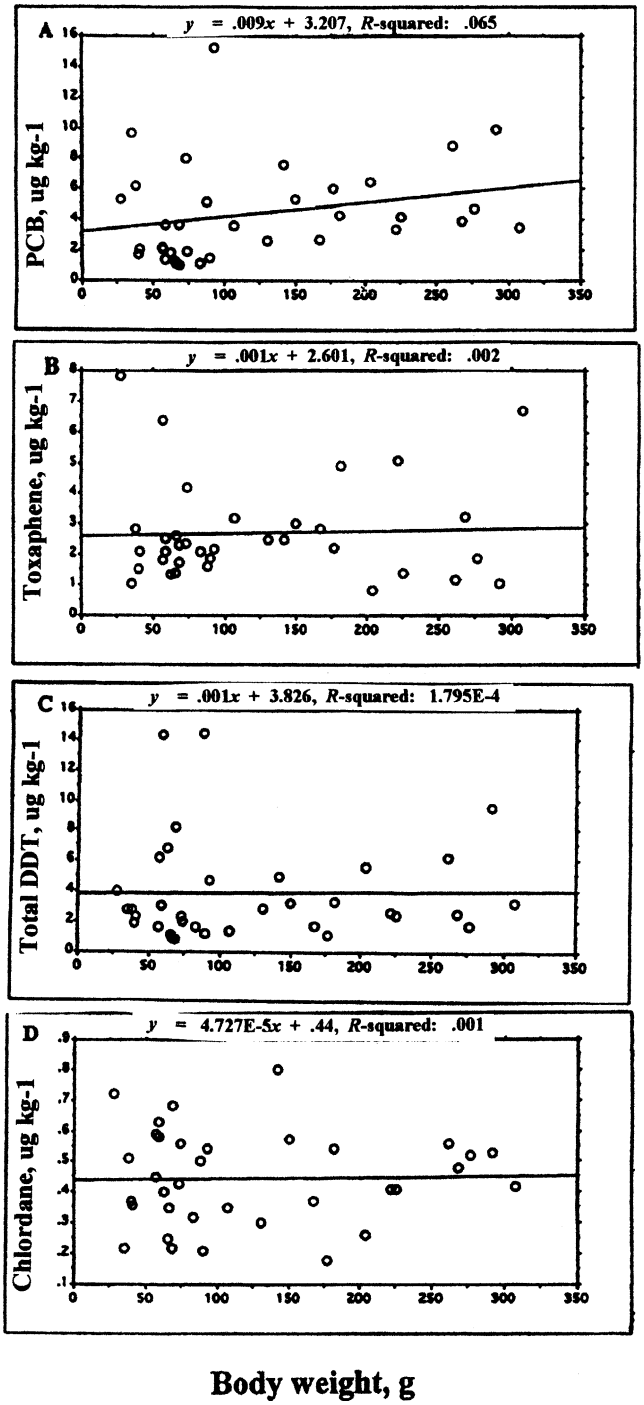

Fig. 4. Relationships between the body weights of all rainbow trout samples (see also Tables 2 and 3) collected from various locations (see Table 1) and residue levels of (A) polychlorinated biphenyl (PCB), (B) toxaphene, (C) total DDT, and (D) chlordane.

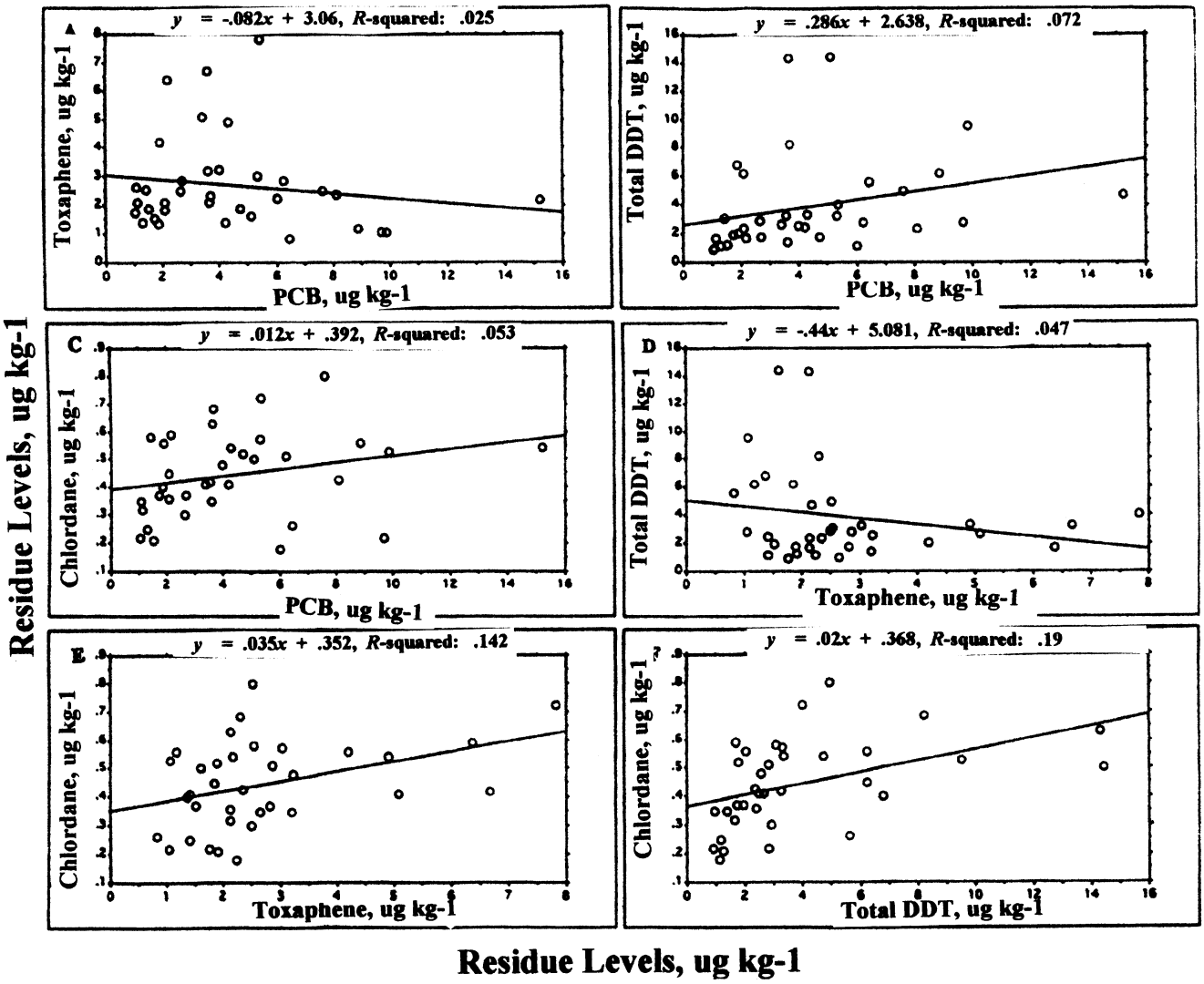

Fig. 5. Paired comparisons of residue concentrations of one organochlorine compound found in all rainbow trout samples (Tables 2 and 3) and those of another organochlorine compound found in the same trout samples. The paired comparisons were (A) toxaphene vs. polychlorinated biphenyl (PCB), (B) total DDT vs. PCB, (C) chlordane vs. PCB, (D) total DDT vs. toxaphene, (E) chlordane vs. toxaphene, and (F) chlordane vs. total DDT.

tude, showing a distinct trend of decreasing concentrations at higher altitudes (covariance = −601.48 and correlation = −0.611). Since this is the key point of this investigation, this set of data (Fig. 7C) was subjected to further statistical regression analysis. When the mean PCB concentration in each location was used in a regression analysis against elevation, the resulting *P* value was 0.018 with $r = -0.939$ (or $r^2 = 0.882$), indicating a strong correlation. Both total DDT (Fig. 7E) and chlordane (Fig. 7F) showed marginal signs of such a trend, but again toxaphene showed no indication of correlation at all (Fig. 7D). We repeated the analysis on correlations among these four organochlorine compounds (Fig. 8) and found that PCB residues are modestly correlated with those of chlordane and total DDT, but not to toxaphene. At the same time, chlordane residues are correlated with both toxaphene (Fig. 8E; covariance = 0.146 and correlation = 0.587) and total DDT (Fig. 8F; covariance = 0.292 and correlation = 0.489), confirming in principle the results of the overall analysis (Fig. 6). As for the influence of west to east locations across the crest of the Sierra Nevada, we found a weak trend of increasing ratio of highly chlorinated congeners to low chlorinated ones in PCB residues from trout samples from the eastern slope (data not shown) as in the case of frog samples (Angermann et al., 2002). However, the number of trout samples analyzed was too small to firmly validate this observation.

DISCUSSION

In the current study, we established that fish even at very high elevation indeed accumulate organochlorine compound residues. The fact that we could find these organochlorine compound residues in all locations we studied is interesting in view of the observation by Dr. J.N. Seiber's group that detectable organophosphate pesticide residues are only infrequently found at high-

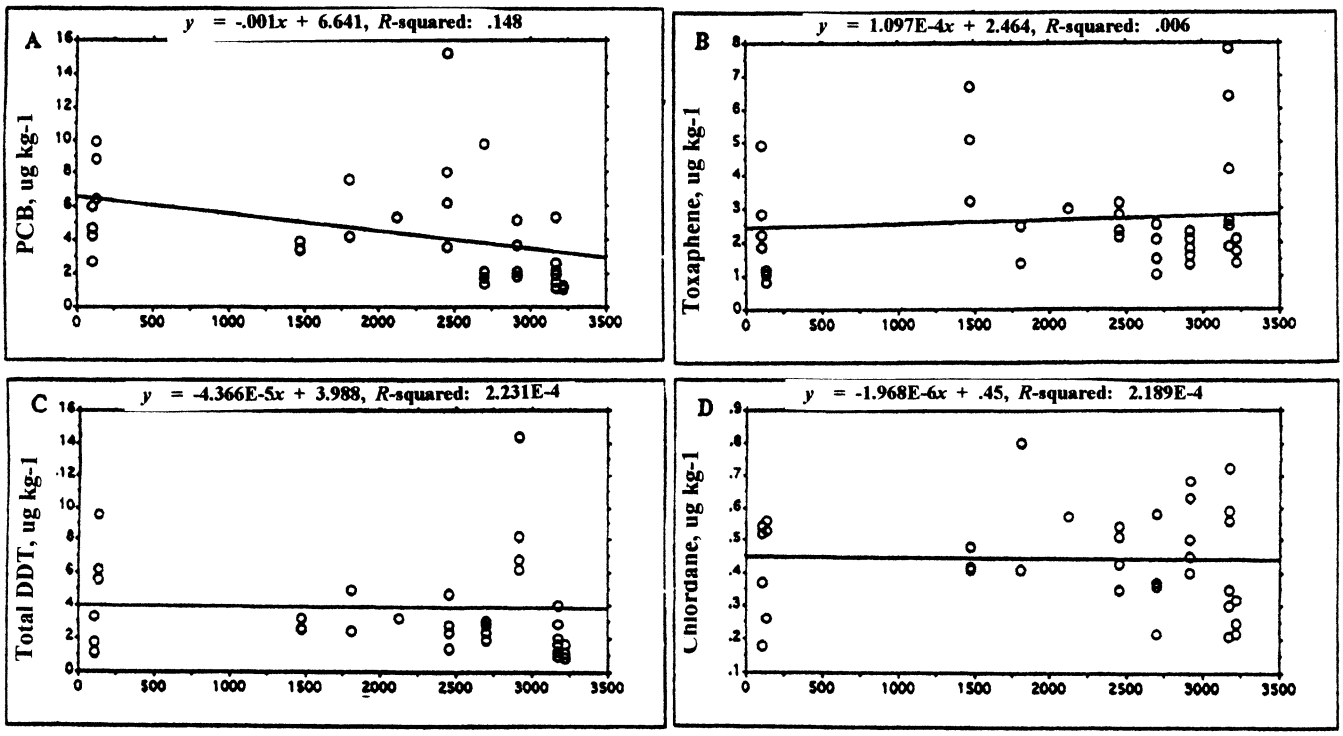

Fig. 6. Relationships between altitude and residue concentrations in all rainbow trout samples (Tables 2 and 3) of (A) polychlorinated biphenyl (PCB), (B) toxaphene, (C) total DDT, and (D) chlordane.

altitude locations in the Sierra Nevada (Aston and Seiber, 1997; Zabik and Seiber, 1993). It was also established in this work that atmospheric transport is likely the main source of pollutant inputs to very high-altitude lakes in the high Sierra Nevada. The ubiquitous distribution of organochlorine compounds over wide areas of the Sierra Nevada supports this conclusion.

At the beginning of this project, we had several concerns about using the fish residue data for direct comparisons among the different sites. The main concerns were that some of the bodies of water sampled in this study are often dissimilar in terms of flow rates, residence time, productivity, vegetative cover, light intensity, and drainage area; all of these factors are known to affect the degree of bioconcentration of organochlorine compounds. To avoid these potential problems, main approaches adopted in this study were to select only similar sized rainbow trout from highly oligotrophic alpine lakes and streams and to analyze the residue data among them only. This species is found throughout the Sierra Nevada region, and moreover, according to Rasmussen et al. (1990), this species gave the least variation in terms of PCB residues among 17 species of fish collected from many locations in the Laurentian region. In view of the controversy on the use of fat-based normalization vs. raw, uncorrected data expressed on a wet-weight basis when comparing PCB residues (Hebert and Keenleyside, 1985), the relationship between the wet weight versus the level of organochlorine compound residues was carefully checked in this study (Fig. 4), but no corre-

Table 5. Summary of the results of ANOVA on differences in residue levels of each organochlorine compound among samples collected from different locations.†

Location‡	PCB§	Toxaphene	DDT	Chlordane
LB vs. HL			*	
DL			*	
A	*		**	*
PL				
RL	**		**	*
BL	**	*	**	
WC	**		**	*
SB			*	
U	**	*	**	
H vs. PL			**	
RL	*			
D vs. PL			**	
U		*		
AQ vs. PL			**	
PL vs. RL			**	*
BL		**	**	
WC			**	
SB	*		**	
U		**	**	
R vs. BL		**		
SB	**			
U		*		
B vs. WC	*			
SB	**			
WC vs. SB	*			
U		**		
SB vs. U	*			

* Significant at the 0.05 probability level.
** Significant at the 0.01 probability level.
† Other combinations yielding no statistically significant differences are not included in this table.
‡ See Table 1 for abbreviations for sample locations. AQ indicates UCD aquaculture.
§ Polychlorinated biphenyl.

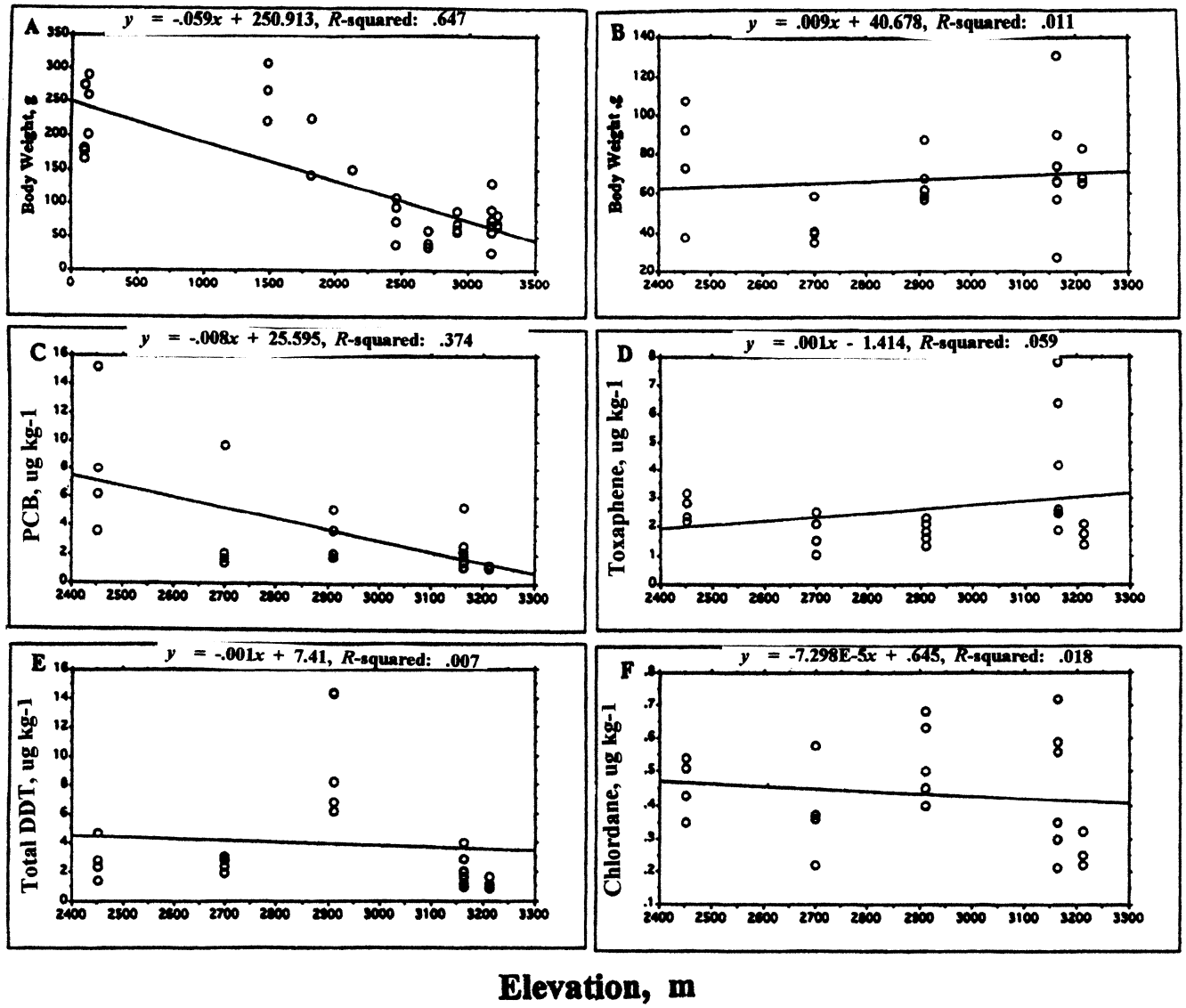

Fig. 7. Relationships between elevation and body weights (A and B) or elevation between residue levels of (C) polychlorinated biphenyl (PCB), (D) toxaphene, (E) total DDT, and (F) chlordane among rainbow trout samples collected from high-altitude locations only (see Table 2).

lations were found on this relationship to wet weight in all cases studied. It was concluded, therefore, that it is better to use only raw, uncorrected values for the comparison among the similar-size fish samples throughout the current study.

The major conclusion of this study is that there is definitely a negative correlation between elevation of the location and the residue concentrations of PCB among those samples (Fig. 7C). The *r* value (the correlation coefficient) of −0.939 is highly significant, since −1 indicates a perfect negative correlation. The square of the *r* value (the coefficient of determination) is 882, meaning that 88.2% of the variability in mean PCB concentrations in the set of data can be contributed to elevation. The use of the mean value of all trout samples from each lake, instead of individual values, in arriving at this high correlation can be justified, since individuals in the same lake could be regarded as statistically pseudoreplicated. This conclusion agrees well with our previous study (Angermann et al., 2002) showing that there is a significant relationship between PCB residue concentrations in tadpoles of Pacific tree frog (*Hyla regilla*) and elevation in the Sierra Nevada range, which considerably overlaps with the area studied in the current project.

The comparison of each of the four groups of organochlorine compounds for their relative abundance among fish samples (Fig. 8) showed that the pattern of chlordane residues in those samples was positively correlated to toxaphene ($R^2 = 0.345$) and total DDT ($R^2 = 0.239$) (i.e., fish that accumulated high amounts of chlordane also accumulated other organochlorine compounds), indicating that some of the processes of chlordane accumulation could be similar to those for toxaphene and total DDT with the probability of 34.5 and 23.9%, respectively. On the other hand, toxaphene residue pattern is not well correlated to PCB or total DDT. This conclusion agrees with the findings of Zell and Ballschmitter (1980), Luckas (1990), and Muir et al. (1990) that toxaphene is a more ubiquitous pollutant than PCBs or

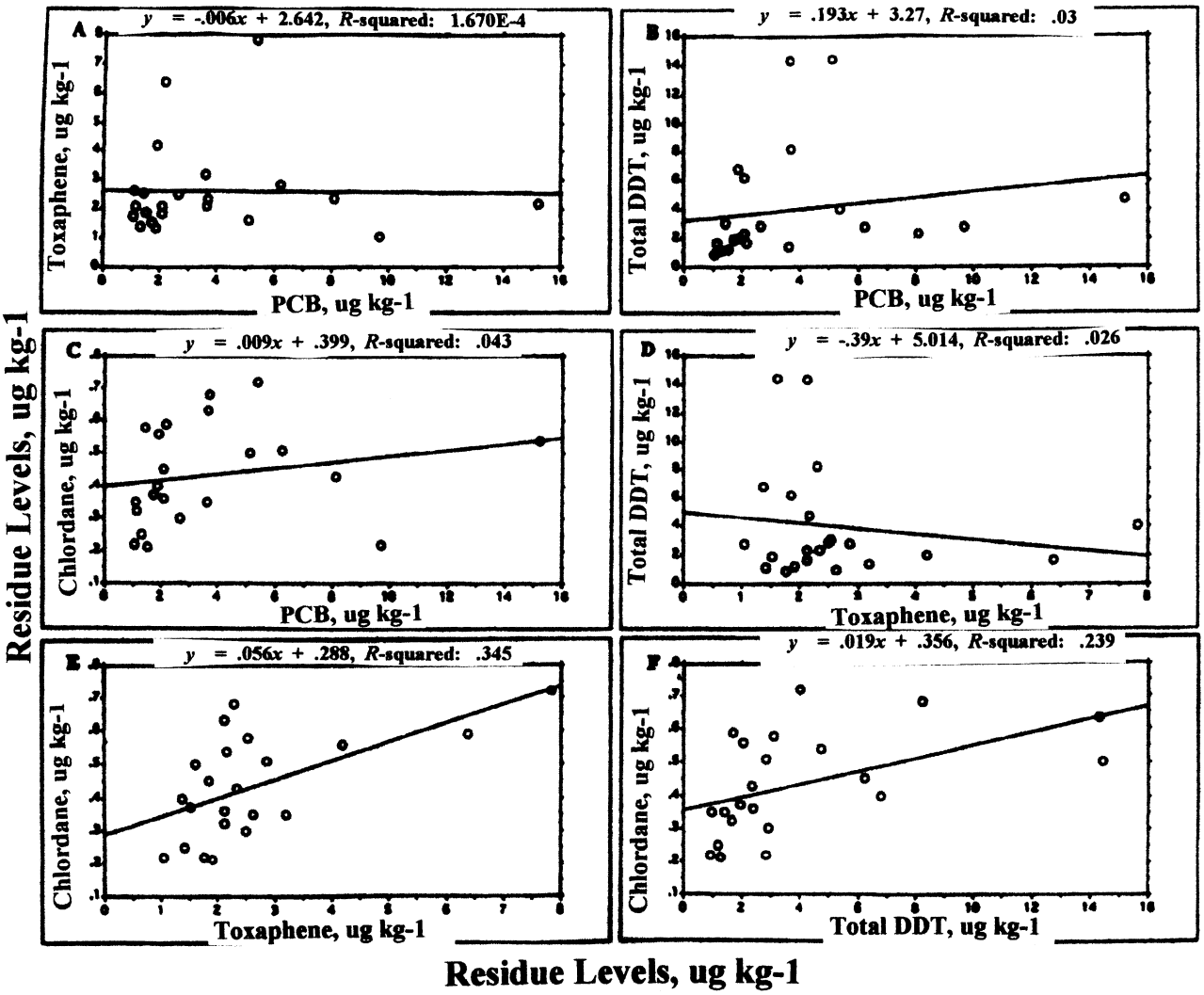

Fig. 8. Relationships between residue levels of one organochlorine compound and those of another organochlorine compound among selected samples of rainbow trout from high-altitude locations (see Table 2). The paired comparisons analyzed were (A) toxaphene vs. polychlorinated biphenyl (PCB), (B) total DDT vs. PCB, (C) chlordane vs. PCB, (D) total DDT vs. toxaphene, (E) chlordane vs. toxaphene, and (F) chlordane vs. total DDT.

total DDT, which tend to be more locally distributed. In this regard, the finding of unexpectedly high total DDT concentrations in Pear Lake requires some explanation. The records show that DDT has been used for insect control in certain areas of the Sierra Nevada (Cory et al., 1970), including this part of Sequoia National Park. Therefore, with regard to the evaluation of long-range transport of pollutants, residual concentrations of total DDT in this particular area of the Sierra Nevada may not be a good indicator.

Finally, it appears to be pertinent to address the question of the influence of physicochemical characteristics on the distribution of these organochlorine compound residues in the Sierra Nevada. Of these four organochlorine compounds, PCBs are known to have the highest volatility, with the vapor pressure values for Aroclor 1254 and 1260 being 7.7×10^{-5} and 4×10^{-5} mmHg, respectively. Chlordane is intermediate in this regard (vapor pressure = 9.75×10^{-6} mmHg), followed by toxaphene (vapor pressure = 6.67×10^{-6} mmHg), DDE (vapor pressure = 6×10^{-6} mmHg), and DDT (vapor pressure = 1.6×10^{-7} mmHg, all values from the National Library of Medicine's Hazardous Substances Data Bank [HSDB]). The general order of tendencies for altitude-dependent decrease in the level of residues in trout among four organochlorine compounds is PCB > chlordane > total DDT > toxaphene. This relationship is roughly in the same order as their volatilities, although the difference between DDE, the main constituent of total DDT, and toxaphene in volatility is insignificant. Such an observation points to the possibility of volatility playing a major role in PCB and probably in chlordane distribution in the Sierra Nevada range.

One possible way to explain such a pattern of distribution affected by the volatility of compounds is that during long-range transportation in the form of air particle–adhered pollutants to high-altitude locations, volatile organochlorine compounds such as PCBs may be more readily lost than less volatile organochlorine compounds such as toxaphene. If this is not the case, how about the other possibility of cold condensation of volatilized compound (i.e., non-particle bound) at high altitude as

the route of transport into the Sierra Nevada? According to Blais et al. (1998), who based their conclusion on the organochlorine compound accumulation in snow packs in the western Canadian Rockies, more volatile organochlorine compound residues accumulate at high-altitude locations because of the cold condensation effect on atmospheric pollutants. Therefore, it is clear that cold condensation is not likely the major mode of transport and depositing of these pollutants to the Sierra Nevada. This consideration further supports our hypothesis that air particulate–bound transport of these pollutants of organochlorine compounds could be the main mode of transport and depositing in this case. According to Cousins and MacKay (2001), partitioning of lipophilic and volatile pollutants into air particles can be explained by the equilibrium between the solubility of these compounds into organic phase of particles, as defined by octanol solubility and vapor pressure–derived air partitioning force (fugacity). In this way one can explain why more volatile PCBs are lost from air particulates at high altitude than less volatile components such as toxaphene and total DDT. Much more work would be needed, however, to firmly establish that air particle–mediated transport is the major mode of introduction of these organochlorine compounds into the Sierra Nevada.

In conclusion, we have established that organochlorine compound residues accumulate in rainbow trout at high-altitude locations in the Sierra Nevada, and that there appears to be negative linear regression of PCB residue concentrations in rainbow trout versus altitude in these mountainous regions. Judging by the fact that these residues are found in very remote locations inaccessible by any motorized vehicles, it is likely that these pollutants have been brought to these sites through atmospheric transportation.

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